REMARKS

Entry of the foregoing, and further and favorable consideration of the claims, in light of the foregoing amendments and the following remarks, are respectfully requested.

By the present amendment, Applicants have deleted material from the specification and have canceled Claims 55-57 without prejudice or disclaimer, purely for the purpose of expediting prosecution. Applicants do not agree with the Examiner's assertions regarding this subject matter, and reserve the right to pursue the same in a continuation or divisional application.

Nonetheless, Applicants maintain that the above amendments to the specification should obviate the issues of priority, oath/declaration, drawings, specification and sequence disclosures. In addition, because Claims 55-57 have been canceled, the rejection of these claims under 35 U.S.C. §112, first paragraph is rendered moot.

The Examiner also rejected Claims 48-51, 53, 54, 56, 57, 59 and 60 as allegedly not being enabled for yeast alpha mating factors other than yeast alpha mating factor pre-sequence ending with arginine operably connected in translation reading frame to DNA encoding the mature human interferon alpha, and not other yeast alpha mating factor prepro sequences. This rejection is respectfully traversed.

The Examiner asserts that "[t]he 'protein' recited in the claims that is secreted or recovered has been assumed to be mature protein since the only protein recited in the claims is encoded by DNA that encodes a 'mature protein'". However, there is no such limitation in the claims currently pending. Indeed, the claims presently under consideration require only that the expression vehicle comprises "a promoter sequence for yeast alpha factor operably connected to a DNA sequence encoding a protein heterologous the yeast" which is transformed with that expression vehicle (Claims 47, 52-53, 58-60) or "a DNA sequence encoding a pre-pro peptide of yeast alpha factor operably connected in translation reading frame to a DNA sequence encoding a protein heterologous to the yeast" (Claims 48-51, 54). The phrase "operably connected" means only that the alpha factor sequences are

connected to the heterologous DNA in such a way that the DNA gets expressed in proper reading frame. Hence, the currently pending claims encompass the production of both mature, and incompletely processed protein. As such, the Examiner's discussion regarding incompletely processed protein is irrelevant to the currently pending claims. Withdrawal of this rejection is therefore respectfully requested.

The Examiner also rejected Claims 47-60 under 35 U.S.C. §102(e) and (g) as being allegedly anticipated by Brake et al U.S. Patent No. 4,870,008. This rejection is respectfully traversed.

The Brake '008 patent refers to an earliest filing date of January 12, 1983. In the *Singh v. Brake* interference to which the Examiner refers, Singh provided evidence which demonstrated that by October 1, 1982, Singh had obtained successful expression of a protein using a construct as recited in the claims (i.e., a DNA sequence encoding a pre-pro peptide of yeast alpha factor operably connected in translation reading frame to a DNA sequence encoding a protein heterologous to the yeast). However, that protein contained N-terminal Glu-Ala sequences. The Board did not agree that Singh obtained properly processed "mature" protein prior to the filing date of Brake, and as such, Brake prevailed in the interference. However, the present claims do not exclude the production of such Glu-Ala-containing proteins, and thus, Singh's October 1, 1982 production of incompletely processed heterologous protein is a reduction to practice which antedates the Brake '008 patent. Copies of the relevant documents and declarations from the interference are attached as an Appendix hereto. Withdrawal of this rejection is therefore respectfully requested.

The Examiner also rejected Claims 47-54 and 58-60 under 35 U.S.C. §102(e) as being allegedly anticipated by Kurjan et al. This rejection is respectfully traversed.

As the Examiner correctly noted, the present claims require that the yeast sequences are in proper translation reading frame to heterologous DNA. The Examiner alleges that Kurjan et al discloses yeast expression vehicles comprising a fusion of a segment of the

yeast alpha factor gene and heterologous DNA. However, the Examiner did not appreciate that Kurjan et al, which contains only prophetic examples, is not enabling for the production of heterologous proteins using yeast pre-pro sequences, because the methods described by Kurjan et al were not sufficient to produce heterologous proteins translated in the proper reading frame.

Submitted herewith is a Declaration by Dr. Ronald Hitzeman, which attests to these facts. Dr. Hitzeman agrees with statements made by Dr. Brake during the prosecution of U.S. Application Serial No. 06/487,950, filed April 25, 1983 to Barr et al. Specifically, Dr. Hitzeman notes the following:

> 7) In my professional opinion, the Kurjan et al. working examples 9a, 9b and 9c are not enabling, and would not teach one of ordinary skill in the art how to successfully accomplish fusion of the alpha-factor gene with a gene coding for a precursor of somatostatin, with a gene for corticotropin, or with a gene for a precursor-enkephalin, nor would the description in these examples, if followed, provide results which would prove the utility of the yeast alpha-factor signal sequence for producing secreted mature heterologous proteins. The technical basis for my opinion is the following discussion of examples 9a, 9b and 9c, pointing out defects which prevent these examples from being capable of achieving their stated purpose.

A. Defects in Example 9a, Kurjan et al.

(1) Treatment of the Kurjan et al. RH1 fragment to fill-in the HindIII cohesive ends (presumably by T4 DNA polymerase or Klenow enzyme) would result in filling-in both the HindIII and EcoRI ends, thus destroying the EcoRI site by not allowing it to be reformed by hybridization to a

complimentary sticky end. Kurjan et al., Example 9a at column 10, line 68 to column 11, line 5 says:

"The cohesive end of the HindIII site of this fragment is filled in enzymatically to produce a fragment denoted RH2 to be ligated to a segment of the somatostatin gene. The RH2 fragment is jointed to a PstI-EcoRI fragment (denoted PE) from the sequence that codes for presomatostatin (Goodman et al.)"

This step permanently destroys this EcoRI site at the same time as it fills in the HindIII site on the R1-2 fragment.

(2) The proposed fusion to the preprosomatostatin cDNA is made at a PstI site which has been modified by poly(dC), poly(dG) homopolymers, the exact length of which was not determined (Goodman et al., P.N.A.S. (1981) 77:5869). Thus, there is only a 1/3 chance of the resulting presomatostatin fusion being in-frame for translation of the correct amino acid sequence. In addition, the polyG sequence and the three 5' nucleotides (AAG) will result in additional residues [$(Gly)_n$-Lys] (where n is polyG nucleotides/3, GGG codes for Gly, AAG codes for Lys) between the alpha-factor leader and preprosomatostatin. Such residues can interfere with secretion of a fusion protein, particularly when additional charged amino acids, such as lysine, are present.

(3) Blunt-end ligation of the fragments provides no way of ensuring the proper orientation in the ligation products and the fragments will be able to form linear and circular concatamers from which the desired products cannot be released by EcoRI digestion (since the EcoRI sites were destroyed by the fill-in reaction in (1) above). Twelve different orientations are equally possible for the blunt end ligation taking two DNA segments at a time. When larger DNA ligation chains form the number of possible orientations becomes

extremely large. The blocked EcoRI site means that it cannot be used to liberate the desired products in order to screen for the correct sequence in order to clone the correct sequence and ensure production of the correct product.

(4) The PstI ends are 3' overhangs and thus cannot be filled in because of the specificity of T4 DNA polymerase or Klenow enzyme. The PstI ends must be rendered blunt by S1 nuclease digestion or by 3' exonuclease activity of T4 DNA polymerase or Klenow enzyme thus removing nucleotides. This is not discussed nor recognized by Kurjan et al.

(5) There are three PstI sites internal to the cDNA sequence, and thus a partial PstI digest must be performed to generate the desired PE fragment. A complete digestion which is presumed from the statement in Kurjan et al., column 11, lines 2-8, would destroy the desired "PE" DNA segment.

(6) Cleavage in the prosomatostatin sequence is at Arg-105, not Lys-105 as stated at line 28, column 11.

B. Defects in Example 9b by Kurjan et al.

(1) The same problems in using the blunt-end RH2 fragment to fuse to the SS fragment will occur as in example 9a, thereby creating a mixture of different species of product with additional amino acids at the amino terminal end.

(2) The 3' SmaI site in the SS fragment ends in the middle of an Arg codon, therefore there will not be a proper translational termination signal in

the fusion protein resulting in translational read-through. Translational read-through of this gene fusion results in the synthesis of a longer protein with an unknown C-terminal extension creating a fusion-protein other than ACTH alone. The extra amino acids might be removed by proteolytic processing as proposed, however it is likely that the additional amino acids would also interfere with proper secretion of the fusion protein and if not removed could destroy the physiological activity of the ACTH.

C. Defects in Example 9c by Kurjan et al.

(1) The problems introduced by blunt-end ligation of the fragments would be even more severe in this example, since at least three fragments are being ligated making the formation of improperly oriented DNA ligations the far majority. There are no measures described which ensure proper orientation of the fragments or which allow release of the desired ligation product from linear ligation products to enable cloning into the appropriate plasmid vector since the EcoRI site has been permanently destroyed by filling in enzymatically.

(2) Again, there is no translational stop codon introduced into the 3' fragment. Therefore, translational read-though will lead to the production of a fusion protein with an unknown C-terminal extension. Such extensions are known to interfere with proper secretion of the fusion protein and also change the character of the product produced from an enkephaline to a fusion peptide product containing an amino acid sequence of unknown length and composition which is not an enkephalin.

In light of the non-enabling nature of Kurjan et al for the production of heterologous proteins translated in proper reading frame, it is not an appropriate anticipating reference against the presently pending claims. Withdrawal of this rejection is therefore respectfully requested.

The Examiner also rejected Claims 47-54 and 58-60 as being allegedly anticipated under 35 U.S.C. §102(e) by Brake et al U.S. Patent No. 4,914,026. This rejection is respectfully traversed.

The Brake '026 patent refers to an earliest filing date of April 7, 1983. As such, the Brake '026 patent is also obviated by the evidence of reduction to practice of an embodiment within the scope of the present claims, prior to that date. Withdrawal of this rejection is therefore respectfully requested.

The Examiner also rejected Claims 55-57 under 35 U.S.C. §103 as being purportedly unpatentable over Kurjan et al in view of Goeddel et al. In light of the cancellation of Claims 55-57, this rejection is moot.

Further and favorable action in the form of a Notice of Allowance is believed to be in order, and is earnestly solicited.

If the Examiner has any questions regarding this amendment, or the application in general, she is encouraged to contact the undersigned directly so that prosecution may be expedited.

Respectfully submitted,
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